Docket No.: 1268-073

PATENT

#27 GP4 6/26/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Shai Yarkoni, et al.

Serial No. 09/147,346 Group Art Unit: 1646

Filed: December 4, 1998 Examiner: L. Helms

For: CHIMERIC TOXINS FOR TARGETED THERAPY

DECLARATION UNDER 37 CFR 1.132

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D. C. 20231

Sir:

I, Haya Lorberboum-Galski, hereby state:

That I am one of the co-inventors of the subject matter of the above referenced patent application;

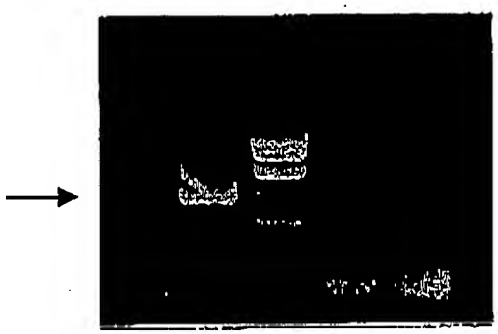
That I am thoroughly familiar with the contents of the above referenced patent application;

That I am thoroughly familiar with the contents of all of the references that have been cited in support of rejections of the patentability of the claims of the above referenced patent application;

That I have conducted, or have had conducted under my supervision and direction, certain tests addressing the patentability of the claims of the above referenced patent application;

That the following Figure 1 shows PRC analysis of the pituitary GnRH Receptor:

1 2 M 3 4



Lane 1: template-cDNA prepared from normal human lymphocytes.

Lane 2: template-cDNA prepared from granulosa cell tumor.

Lane 3: template-cDNA prepared from normal human granulosa cells.

Lane 4: template-cDNA prepared from normal human pituitary.

M=marker.

Arrow indicates the expected PCR product of 991 bp.

Primers used for the PCR reaction 5 GCTTGAAGCTCTGTCCTGGGA-3 (sense starting from nucleotide -25, according to Kadar T. et al, 1992, Biochem. Biophs. Res. Comm. 189, 289-295) and 5 -GATAAGTGGATCAAAGCATGG-3 (antisense starting from nucleotide 946). The reaction mixture was incubated in a DNA thermal cycler (MJ Research, Inc, USA.) for 39 cycles. Each cycle consisted of 1 min. at 94°C, 1 min. at 60°C and 2 min. at 72°C.

That the following Table 1 shows the effect of GnRH-PE66 on various cells:

	protein synthesis, % of the control	protein synthesis, % of the control	protein synthesis, % of the control
	10 (ng protein)	50 (ng protein)	500 (ng protein)
Granulose cell tumor	102	99	104
Caco2	42	6	1

Cells (10^4 in 0.2 ml culture medium) were seeded in 96-well microplates, and 20 hrs later various concentrations of GnRH-PE66 were added. After an additional 24 hrs incubation, [^{3}H]leucine (1 μCi (37 kBq)/well) was added overnight. The plates were then stored at -70°C for several hours, followed by quick thawing at 37°C. The cells were harvested on filters and the incorporated radioactivity was measured in a β counter. The results are expressed as the percentage of protein synthesis of control cells not exposed to chimeric proteins;

That therefore these data show that the mechanism of action of the molecules of the prior art and the molecules of the protein of this invention are different;

That the mechanism of action of the GnRH-toxin conjugate for treatment of hormone-dependent types of cancers is completely different from the mechanism of the action of the instant claimed chimeric protein. The GnRH-toxin conjugate (prior art) is not proposed to work directly on the cancer cells but rather it has been proposed that it works by an indirect effect. Because the conjugate is believed to "be specifically targeted to the gonadotropin-secreting cells of the anterior pituitary gland...[these are] the only cells to which the gonadotropin-releasing hormone portion of the conjugate will bind...and thereby eliminate the gland s ability ...to secrete LH and FSH and thus is rendered sterile.." Sex steroid-dependent tumors which respond to such hormonal manipulation are thus believed to have their growth controlled because of lack of secretion of steroid hormones in a sterilized animal/human carrying such tumors;

That, on the other hand, the GnRH-PE chimeric protein of this invention works through its direct action on the tumor cells and the cell killing that results because, as shown by the above detailed data, adenocarcinomas express GnRH binding sites; the

GnRH-PE chimeric proteins bind to these binding sites, thus allowing the internalization of the chimeric protein directly into the adenocarcinoma cells. Upon internalization into the cancer cells, the Pseodomonas Exotoxin-killing moiety, inhibits protein synthesis (the natural activity of the toxin), which leads to the death of the cells. Thus, GnRH-PE chimeric proteins directly act and kill the cancer cells (adenocarcinomas).

That the applications of the GnRH-toxin conjugates (prior art) and GnRH-PE chimeric proteins (claimed invention) molecules vary;

That the GnRH-toxin conjugates (prior art) have been suggested mainly for the sterilization of animals (veterinary medicine). In human medicine these reagents are suggested to be used for the following limited purposes; to control fertility (to achieve infertility effects), to treat sex steroid-dependent tumors such as breast and prostate cancers (the only mentioned one!) and for the treatment of endometriosis.

That, as demonstrated by the instant reported data, the GnRH-PE chimeric protein of this invention is a cytotoxic agent for a wide verity of cancers, most surprisingly, even for cancers originated in non-hormone dependent tissues such as colon carcinoma, lung carcinoma, renal carcinoma hepatocarcinoma and more.

That the main applications of the instant claimed chimeric protein are:

malignant carcinomas, including non-hormone dependent cancers (a wide verity of adenocarcinomas); and

benign tumors of the uterus and hyperplasia, including uterine lyomyoma, endometriosis, benign prostate hyperplasia, breast polycystic disease and pituitary adenoma.

That the instant data show that the GnRH-based chimeric proteins of this invention specifically target and kill only adenocarcinoma cells;

That these results verified the inventors earlier findings that GnRH-based chimeric proteins very specifically target and kill only adenocarcinoma cells.

That a wide variety of killing proteins were used in the testing of the chimeric proteins (such as the PE toxin) or human apoptotic proteins (such as proteins of the Bcl-2 family-Bax, Bak, Bik, or the DNase DFF40) of this invention, and all (when fused to the GnRH targeting moiety), irrespective of whether they were bacterial or human pro-apoptotic proteins, caused cell death of only adenocarcinoma cells. It is also pointed out

that the various killing moieties used not only differ in their origin but also in their size.

That these reported data further highlight the fact that the GnRH sequence is, indeed, responsible for targeting the various chimeric proteins to the specific intended target cells, and any protein fused to GnRH in the form of the chimeric protein of this invention, will enter the cell via the GnRH-binding sites.

That the following tables demonstrate the activity and specificity of another GnRH-based chimeric protein GnRH-DFF40, in which an identical GnRH targeting moiety is fused to the human pro-apoptotic protein DNA Fragmentation Factor 40 (DFF40):

Table 2: Effect of GnRH-DFF40 on Various Human Cell lines

	Cell lines	Cell type	Live cells (% of control)[a]
Target cell lines	293	Renal cell adenocarcinoma	42±13
	Colo 205	Colon adenocarcinoma	25±10
	DLD-1	Colon adenocarcinoma	34±6
	HCT-15	Colon adenocarcinoma	40±1
	SW-48	Colon adenocarcinoma	41±2
	MCF-7	Breast adenocarcinoma	41±11
	HepG2	Hepatocarcinoma	50±4
Nontarget cell lines	T24A	Bladder carcinoma	96±7
	J82	Bladder carcinoma	97±5
	A204	Rhabdomyosarcoma	99±1

[a] ± Indicate the standard deviation of 3-4 experiments, preformed using 2-3 different chimeric protein preparations. Partially purified GnRH-DFF40 preparations were used in all experiments. Controls received PBS in equal volumes.

Table 3: Effect of various chimeric proteins on 293 renal carcinoma cells

	Chimeric protein	Target	Live cells (% of control)
Positive control	GnRH-DFF40	Adenocarcinoma	42
	L-GnRH-PE66	Adenocarcinoma	13[a]
	GnRH-BIK	Adenocarcinoma	47
Negative control	IL2-BAX	Activated B,T cells	97
	BPP-BAX	MBP-T cells	140
	Fc-BAX	Mast cells	99

[a] L-GnRH-PE66 was tested using a highly purified protein (1); the other chimeric proteins were only partially purified preparations.

That these data clearly support the assertion that the chimeric fused proteins of the instant invention are distinctly different from the conjugated proteins of the prior art and that the instant chimeric proteins possess unusual, unexpected and unobvious properties as compared to the conjugated proteins of the prior art.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Lorberboum-Galski H.

Haya Lorberboum-Galski

Executed this 11day of June, 2003